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(54) DNA sequences encoding modified retroviral gag polypeptides and vaccines containing them or aggregates thereof

Für abgeänderte retrovirale GAG-Polypeptide kodierende DNA-Sequenzen und diese enthaltende Impfstoffe oder Aggregate davon

Séquences d'ADN codant pour des polypeptides rétroviraux modifiés et vaccins les contenant ou agrégats de ceux-ci

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- ANALYTICAL BIOCHEMISTRY, vol. 161, no. 2, March 1987, pages 370-379; R.L. SHOEMAN et al.: "Comparison of recombinant human immunodeficiency virus gag precursor and gag/env fusion proteins and a synthetic env peptide as diagnostic reagents"

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Description

[0001] The technical problem underlying the present invention is to provide DNA sequences encoding modified retroviral p55-gag polypeptides that can be used for the immunization of mammals against retroviral diseases. This invention particularly relates to a newly designed recombinant HIV subunit vaccine, produced in appropriate expression systems like e.g. recombinant baculo- or vaccinia viruses in insect cells or mammalian cells, respectively. The invention is based on the HIV-1 group specific antigen p55-gag constituting the viral core and its particle forming capacity, when produced in eukaryotic cells. The immunogenicity of the premature 90-110 nm p55-core particles can be extended by inserting well characterized epitopes of other HIV-1, HIV-2 or SIV derived reading frames (e.g. env, nef, pol) into 3 different regions of the gag coding sequence that were selected by computer assisted analysis of the p55 secondary structure. To demonstrate the universal principle of stable expression and enhanced immunogenicity of recombinant p55/gp120 particles, sequences exemplified by the gp120 CD4-binding region, a consensus sequence of the major gp120 neutralizing epitope V3 and a sequence of gp41 ("fusogenic region") were inserted into the coding sequence of the p55 carrier protein and the resulting constructs expressed by recombinant vaccinia- and baculo viruses. Recombinant p55/gp120 vaccinia viruses can be applied directly as live vaccines or as source of protein antigen. Recombinant baculoviruses are used for efficient production and purification of the particular and chimeric p55/gp120 subunit vaccines only.

[0002] With regard to the ability of HIV, to persist latently in infected cells, the elimination of free virus by neutralizing antibodies as well as the clearance of infected cells by antibody dependent cell cytosis (ADCC) and cytotoxic T-cells is of decisive significance for disease progression. In order to learn more about how the immune system deals with free virus or HIV infected cells, many labs focused on the identification and characterisation of B- and T-cell epitopes, including all reading frames. The role of humoral immune response is crucial in HIV infection. During all stages of disease progression, except the final stages of AIDS, antibodies to almost all HIV proteins can be detected (Vomhagen et al., 1989). Neutralizing epitopes directed to the outer structural proteins gp120 (Goudsmid et al., 1988; Parker et al., 1988), gp41 (Chan et al., 1986) and to the inner structural proteins p17 (Papsidero et al., 1989) and p24 (Wolf et al., 1990) were described, that might play a role in the elimination of free virus. Antibody dependent cell mediated cytotoxicity (ADCC; Lyerly et al., 1987, 1988) may be important for elimination of HIV infected cells. One such ADCC epitope seems to be located in the C-terminal part of gp120. However, antibodies are also known to influence progression of disease negatively (Fc and/or complement mediated antibody enhance-

ment; Takeda et al., 1989, Homsey et al., 1989, Robinson et al., 1989). The proliferation of T4 cells can be group specific and was proven to be directed towards distinct env, gag and pol epitopes by application of synthetic peptides (Schrier et al., 1989). T-cell mapping with recombinant gp120 vaccinia viruses identified four T-helper epitopes on the gp120 (Berzofsky et al., 1988, Krohn et al., 1988). Moreover, three p24 specific CD4 positive T-cell lines could be established. MHC class I

- 5 restricted CTL epitopes were defined for env (Takahashi et al., 1988, Siciliano et al., 1988), gag (Nixon et al., 1988) and pol proteins (Walker et al., 1988, 1989). CTL mediated lysis is of outranging importance for the elimination of infected cells. Other virus systems (Influenza-, Rabies-, Cytomegalovirus) indicated, that non structural proteins are capable to generate immunity by induction of cytotoxic T-cells, and that a priming of the immune system is achieved, which upon challenge, causes a more effective response even towards other viral proteins (Milich et al., 1988).
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[0003] The use of total HIV or of intact HIV encoded genes can not exclude adverse effects upon immunization. Amongst such negative effects are antibody enhancement, autoimmune reactions, induced tolerance or allergic side effects.

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[0004] Despite initial encouraging in vitro results immunization with complete gp160 or subunits does not seem to be sufficient to induce protective immunity. The neutralizing properties of the induced immune response was restricted to the same isolate, used for immunization (type-, not group specific; Nara et al., 1988). Additionally, it was shown, that HIV can still amplify in gp160 immunized animals after challenge (Hu et al., 1987). Several reasons might explain the failure of protective immunity:

1. The decisive epitopes of gp120 vary faster than the elimination by antibodies.
2. The virus spread is mediated at the beginning of the infection by direct cell-cell contact. A humoral immune response is not sufficient and a cellular response to gp120 eventually not sufficient.
3. Enhancing antibodies may negatively influence disease progression. Complement dependent and Fc-mediated enhancement of HIV infection has been shown in different in vitro studies (Takeda et al., 1988, Homsey et al., 1989, Robinson et al., 1989).
4. Epitopes which mimic products of normal cellular genes elicit immune responses, which attack normal cells (Reiher et al., 1986, Golding et al., 1988, 1989).
5. Immunosuppressive effects of the HIV envelope: free gp120 binds with high affinity to the CD4 recep-

tor molecule of target cells and labels them for ADCC(Lyerly et al., 1988) and CTL attack in vitro (Siliiano et al., 1988, Lancaveccchia et al., 1988). Furthermore, there is evidence that binding of gp120 to the CD4 molecule interferes with the region of the CD4 receptor, which is necessary for the interaction with MHC class II molecules (Weinhold et al., 1989, Clayton et al., 1989). Moreover, regions of gp 120 homologous to those of the transmembrane glycoprotein of human type C retroviruses known to be highly suppressive for macrophage function are also present in HIV gp41 (Clanciola et al., 1988).

[0005] Thus, the technical problem underlying the present invention is to provide DNA sequences encoding a modified retroviral p55-gag polypeptide that can be used for the immunization of mammals against retroviral diseases.

[0006] The solution to the above technical problem is achieved by providing the embodiments characterized in the claims.

[0007] Accordingly, the present invention relates to a DNA sequence encoding a modified retroviral gag polypeptide as characterized in the claims, said gag polypeptide containing at least one amino acid sequence representing a non-gag polypeptide antigenic determinant, wherein the DNA sequence is derived from any of the retroviruses HIV-1, HIV-2 or SIV, wherein the gag polypeptide is p55, wherein said amino acid sequence substitutes a region(s) of said gag polypeptide, said region(s) being capable of presenting the inserted amino acid sequence to the immune system so as to elicit an immune response or is fused to the COOH terminus of said gag polypeptide, and wherein the region referred to above is located at amino acid positions 99 to 154, 211 to 241, or 436 to 471 or at any combination thereof.

[0008] Such an immune response either is a humoral or a cell-mediated immune response. Such immune responses involve the B-cell and/or T-cell compartment.

[0009] The term "of presenting" refers to the availability of an antigen for immunorecognition in a way to initiate primary and /or recall immune response.

[0010] The regions into which said amino acid sequences are inserted or which are substituted by said amino acid sequences are selected according to the following criteria:

- (a) hydrophilicity,
- (b) flexibility and
- (c) surface probability of the deleted region.

[0011] In a preferred embodiment of the present invention the above referred to amino acid sequence replaces gag polypeptide sequence positions.

[0012] In another preferred embodiment of the present invention the DNA sequence encoding said additional amino acid sequence inserted into the gag

polypeptide is inserted via a linker. Such linkers are conventional linkers which may contain several restriction enzyme cleavage sites permitting the incorporation of DNA sequences encoding the above mentioned additional amino acid sequence according to conventional methods.

5 [0013] In preferred embodiments of the present invention the non-gag polypeptide antigenic determinant is:

- 10 (a) at least a part of the CD4-binding domain of gp120;
- (b) at least a part of the variable region 3 of gp120; or
- (c) at least a part of a conserved region of gp 41.

[0014] Particularly preferred parts of the CD4-binding domain of gp120 are derived from the V3 region thereof and include the subregions and derivatives of said V3 region which are described in detail in the European Patent Application claiming priority of EP 90 10 5313.2

20 [0015] The above-mentioned parts of the proteins are selected regions thereof which are capable of inducing an immune response to the naturally occurring virus. Preferably they are capable of inducing the formation of antibodies which react with the naturally occurring virus or a cell-mediated immune response to infections with said virus.

[0016] In another embodiment the invention relates to 30 recombinant vectors containing any of the DNA sequences referred to hereinbefore.

[0017] Said recombinant vectors include conventional plasmids which may be used for the cloning or for the expression of the DNA sequence in a host cell. In case of expression plasmids the DNA sequence is preferably combined with a promoter sequence controlling its expression in suitable host cells.

[0018] In a further embodiment the invention relates to 40 recombinant viruses containing a DNA sequence as referred to hereinbefore. Preferably, this recombinant virus is derived from vaccinia virus.

[0019] The invention also relates to host organisms which are transfected by said recombinant vectors or recombinant viruses.

[0020] Furthermore, the present invention relates to 45 a modified retroviral gag polypeptide which is encoded by any of the DNA sequences referred to above.

[0021] The present invention additionally relates to aggregates which are in the form of particles.

[0022] In a further embodiment the present invention relates to a method for the production of a modified retroviral gag polypeptide as characterized hereinbefore said method comprising cultivating a host organism as mentioned above on the suitable conditions and recovering the expression product from the medium.

55 [0023] Its a further specific embodiment of the invention to produce said gag particles in a baculo virus dependent expression system in *Spodoptera frugiperda*

insect cells and, using stably transfected cell lines, in *Drosophila Schneider* cells.

[0024] Finally, the invention relates to a vaccine containing a recombinant virus as described hereinbefore, a polypeptide forming particles as described hereinbefore. In preferred embodiments these vaccines also contain a pharmaceutically acceptable carrier and/or diluent.

[0025] To develop a recombinant designed vaccine, that is capable to induce protective immunity to HIV infection, detailed knowledge about antigenicity and variability of structures, that are involved in virus adsorption and penetration are necessary. Few alternative approaches are based on HIV derived gp120 depleted or partially depleted particles or purified gag protein.

The following rationales are bases of our present invention

[0026]

1. Because of the observed variability of HIV large parts of immunologically relevant gene products must be included.

2. Epitopes known to elicit enhancing antibodies and epitopes known to mimic cellular proteins, should be excluded.

3. Those regions, that induce T-cell immunity and that are targets off ADCC, must be taken into consideration as components of the vaccine.

4. The vaccine product should be generated as a particulate antigen to act as immunogen in a stable form without application of additional adjuvants.

5. Alternatively recombinant viruses such as *Vaccinia*, *Herpes simplex* or *Adenovirus* can be used to express these components directly in infected cells.

[0027] The possibility to produce premature p55-core particles in eukaryotic expression systems enables us to design a subunit vaccine, that performs the above mentioned postulates. Autologous p55-core particles are expressed by our group and others in the vaccinia and baculo expression system. As known from other particular subunit vaccines (e.g. Hep. B 22 nm particles), particulate structures show an intrinsic adjuvant potential which is used in our invention.

Production of recombinant p55/gp120 hybrid particles according to the invention

[0028] As a consequence of all findings, we used p55-core particles as an autologous carrier for appropriate epitopes out of the context of other reading frames.

5 1) p55-gag was cloned into a comercial pUC-vector and expressed in *E. coli* strain JM109. For cloning of the authentic p55 protein without untranslated sequences the PCR (Polymerase Chain Reaction) was used. Following expression of p55 in eukaryotic systems was achieved by recombinant *Vaccinia* and *Baculo* viruses. The formation of p55 particles was proven by sucrose gradient sedimentation and electron microscopy.

10 2) The identification of appropriate positions in the p55-core protein for the insertion of epitopes from other reading frames was performed with computer assisted analysis of the p55 secondary structure (Wolf et al., 1988). By this approach we predicted four regions within the p55-core protein being exposed on the surface of the protein.

15 3) For the development of the recombinant p55-core/gp120-env sequences a polylinker was synthesised. This linker sequence contained all restriction sites, which were necessary for the development and following expression of three distinct p55 deletion constructs each missing a region coding for 30-45 amino acids. By this compatibility of all deletion constructs for insertion of foreign sequences, for subcloning and following expression in eukaryotic vector systems was achieved.

20 4) In a first approach, three epitopes from the HIV-1 gp120-env reading frame were selected for insertion in the p55 deletion constructs:

The CD4 binding region

[0029] Adsorption of free virus and HIV infected cells to CD4 target cells is directed by the binding of the viral external membrane protein gp120 to the cellular membrane protein CD4. The CD4 receptor molecule is mainly expressed on T4-lymphocytes and cells of the monocyte- and macrophage lineage. The region being responsible for the interaction with the CD4 receptor is located in the C-terminal part of gp120 (aa413-451) and well conserved in different HIV isolates (Kowalski et al., 1987, Lasky et al., 1987). Although the CD4 binding region seems not to be immunogenic in humans in natural infection, it was shown that the insertion of this region into the VP1 surface structural protein of poliovirus induces neutralizing antibodies in infected animals. Moreover, monoclonal antibodies, directed towards this sequence have neutralizing capacity and inhibit syncytia formation in vitro by blocking CD4 dependent attachment of free virus or HIV infected cells to the target cells (Sun et al., 1989).

GPGR-V3-loop of gp120

[0030] The hypervariable V3-loop of the gp120 was

described from our group as the only hypervariable region without consensus sequence for N-glycosylation and discussed in its immunological importance (Modrow et al., 1987). This region was identified to be the major HIV-1 neutralizing epitope and could be correlated with a 24 aa sequence (aa 302-326). A 6 amino acid peptide is sufficient to induce neutralizing antibodies. This sequence contains a 4 aa beta turn motif, GPGR, that is highly conserved in different isolates (Palker et al., 1988, Goudsmid et al., 1988). Flanking amino acids seem to be critical for antibody binding and type specific virus neutralization. A 14 aa sequence (315-329) was characterized to be an ADCC target region. Moreover, it could be shown, that MHC class I restricted cytotoxic T-cells recognize a 18 aa peptide of the V3 region in the mouse model (Takahashi et al., 1988). How anti-V3-neutralizing antibodies actually inhibit infection and syncytia formation in vitro (Skinner et al., 1988) is not clear to date. Possible hints are given by recent results, that indicate a processing of gp120 under certain assay conditions in 2 polypeptides (50 KD, 70 KD). The cleavage site was mapped directly to the conserved sequence GPGRA of the V3-loop. The processed gp120 is still able to bind to the CD4 molecule, but can't be any longer recognized by anti-V3-neutralizing antibodies. Trypstatin, a new Kunitz-type protease inhibitor with a high sequence homology to the gp120-V3 region, inhibits the described protease activity and the following syncytia formation. The V3 dependent, postbinding processing event seems to be a predisposition for the fusion of the outer viral membrane or the membrane of HIV-1 infected cells with a CD4 target cell (Hattori et al., 1989). This essentially could explain the inhibitory effect of anti-V3-neutralizing antibodies. Recent evidence indicates that the deletion of this loop from an infectious clone abolishes infectivity of the virus.

The gp41 conserved region

[0031] The gp41 mediates the membrane fusion of bound HIV with the CD4 target cell and the syncytia formation of infected with uninfected cells (Kowalski et al., 1987, Mc Clure et al., 1988). Monoclonal antibodies raised to a gp41 group specific antigen neutralize a panel of different HIV-1 isolates in vitro. Insertion of the gp41 neutralizing epitope into the poliovirus VP1 and following immunization of experimental animals resulted in the induction of anti-gp41-neutralizing antibodies, that inhibited syncytia formation (Evans et al., 1989).

[0032] The CD4 binding region and the gp41 fusogenic region were amplified and subcloned into the p55 deletion constructs using PCR reaction. The consensus sequence of the major gp120 neutralizing epitope V3 was chemically synthesized and also inserted into the p55 deletion clones. To determine correct expression of the designed p55/gp120 fusion proteins, the recombinant proteins were produced in E. coli and verified by Western blot analysis.

5) After subcloning of the final recombinant p55/gp120 constructs into eukaryotic transfer vectors these hybrid genes were expressed in vaccinia- and baculo virus expression systems. The correct expression was verified by Western blot analysis and immunofluorescence. By electronmicroscopy particle formation was studied.

Brief description of the figures

[0033]

Fig.1: Description of the cloning procedure to the E. coli expression clone pUC8p55

Fig.2: Expression of p55 (v-p55) and p55 in combination with the HIV protease (v-p55prt) by recombinant vaccinia viruses. CV1 cells were infected with 10 pfu of p55 recombinant vaccinia virus. The cells were harvested after 36 hours (v-p55) and at different intervals after infection and resuspended in boiling mix. The infected cells were assayed by conventional Western blot analysis as follows: Cell lysates were separated in a 17.5% PAGE and blotted on nylon membranes (Schleicher and Schuell). 55KD and 41KD recombinant proteins were detected by monoclonal antibodies directed to p24 (mab 13/5) and to p17. As proven by the experiment, the HIV-1 protease has no influence on the expression of p55 in the vaccinia expression system. As shown by the reaction of the shortened 41 KD product with the anti-p17 monoclonal antibody, at least part of the shortened product shares the N-terminal sequences with the complete 55KD protein.

Fig.3: Expression of p55 by recombinant baculoviruses (b-p55). Spodoptera frugiperda cells were infected with 10 pfu of said virus. Infected cells were harvested daily until day 5. Cells from day 1-5 and culture supernatant from day 5 were resuspended in boiling mix and assayed by conventional Western blot analysis. Recombinant proteins were specifically detected by monoclonal antibodies directed to p24.

Fig.4: 2ml of culture supernatant of b-p55 infected cells, harvested 5 days after infection, were assayed by sucrose density sedimentation (2,5 hours, TST 41.14; 28000 rpm). 19 fractions (600 µl) were collected and 20 µl of each fraction were analyzed by conventional Western blot analysis us-

ing p24 specific monoclonal antibodies for the detection of the recombinant protein. As proven by following negativ staining, premature p55 particles accumulated in fraction 17 at a sucrose density of 45%. 5

Fig.5: Spodoptera frugiperda cells, infected with 10 pfu b-p55, were fixed 5 days after infection in 2% glutaraldehyd and assayed for particle formation by ultrathin section electron microscopy. Premature 90-110 nm p55 particles were detected budding from the cytoplasmic membrane. 10

Fig.6: 4 regions were predicted by computer assisted analysis of the p55 secondary structure to be exposed epitopes on the surface of the core protein. Those regions were deleted and supplemented by small polylinkers, selected for simple integration of foreign epitopes. 15

Fig.7: synthetic primer sequences used in PCR reaction, small synthetic linker fragments to make the p55 deletion constructs compatible to each other with regard to the reading frame for the insertion of foreign epitopes and a synthetically produced epitope of the HIV-1 gp120. 20

a) synthetic polylinker for the construction of the pUC8 derived plin8-vector.
b) synthetic primers used for amplification of the 3 part of the p55 deletion construct-4. 25

c) synthetic primer sequence necessary for amplification of the HIV-1 derived CD4 binding region of gp120.
d) synthetic primer sequence used for amplification of the HIV-1 derived gp41 fusogenic region.
e) sequence of a chemically synthesized consensus region of the major HIV-1 neutralizing epitope V3. 30

Fig.8: cloning procedure for development of the plin8p55 1 construct. 35

Fig.9: cloning procedure for development of the plin8p55 2 construct. 40

Fig.10: cloning procedure for development of the plin8p55 3 construct. 45

Fig.11: cloning procedure for development of the plin8p55 4 construct. 50

Fig.12a: Expression of three different plin8p55 deletion constructs (plin8p55 2, 3, 4) next to the expression of the respective clones with the inserted consensus sequence of the major HIV-1 neutralizing epitope V3 (plin8p55V3-2,-3,-4). Recombinant bacteria were grown to an optical density of 0,6, induced for 2 h by 2 mM IPTG, lysed in boiling mix and characterized by conventional Western blot analysis. The recombinant p55V3 fusion proteins were specifically detected by monoclonal antibodies directed to p24 (a) and by human serum pool (b). 55

Fig.12b: Expression of three different plin8p55 deletion constructs (plin8p55 1, 2, 3, 4) next to the expression of the respective clones with the inserted CD4-binding region of the HIV-1 gp120 C3 region (plin8p55CD4-1,-2,-3,-4). Recombinant bacteria were grown to an optical density of 0,6, induced for 2 h by 2 mM IPTG, lysed in boiling mix and characterized by conventional Western blot analysis. The recombinant p55CD4 fusion proteins were specifically detected by monoclonal antibodies directed to p24. 60

Fig.13: Expression of three different p55/V3 fusion proteins (p55/V3-2,3,4) in E. coli after induction with 2mM IPTG at an O.D. of 0,6. The recombinant p55V3 fusion proteins were specifically detected by monoclonal antibodies directed to p24 (a) and (c) to the V3 epitope of a foreign isolate (IIIb). A polyclonal serum raised against a synthetic peptide, representing the original sequence of the V3 consensus sequence, detected the p55/V3 even better than the IIIb-peptide derived monoclonal antibody. The fact, that neither the monoclonal antibody, nor the polyclonal V3 specific serum detect the p55V3-2 protein indicates, that the antigenic context of the V3 region is important for immunological detection. 65

Fig.14: CV-1 cells were infected with 10 pfu v-p55V3-3, harvested 2 days after infection and characterized by conventional Western blot analysis using monoclonal antibodies directed to p24 and the IIIb derived V3 region. 70

[0034] The examples illustrate the invention. 75

Example 1: Expression of p55 in E. coli (standard methods: Sambrook et al., 1989)

[0035] Before p55 could be produced in eukaryotic systems, it had to be cloned and expressed in E. coli. For preliminary expression, the pUC-vector system was chosen. To clone the 5' sequence of the core protein precisely, without longer untranslated sequences, we inserted a synthetic 62 bp oligonucleotide, encoding the N-terminal 15 aa of p55, in frame with the lacZ alpha fragment into the Bam HI/Hind III site of the pUC 8 vector. Two 3' in the oligonucleotide localized restriction sites, Clal and Sall, enabled us to complete the gag reading frame by insertion of a 1694 bp p55-Clal/HincII fragment; fig.1). The expression of the recombinant protein in E. coli after induction with 2mM IPTG resulted in the production of a 55 KD protein proven by conventional Western blot analysis using monoclonal antibodies.

Example 2: Expression of p55 by recombinant vaccinia viruses

[0036] The BamHI/Sall fragment encoding the total p55 reading frame, could be directly subcloned into the vaccinia pAvB transfer-vector and recombined into vaccinia viruses. Expression products were analyzed by conventional Western blot analysis using anti-p24 antibodies (fig.2).

Example 3: Expression of p55 by recombinant baculo viruses

[0037] In order to express the core protein by recombinant baculo viruses, it had to be subcloned twice: The BamHI/Sall fragment had to be inserted into the Bam HI/Sall site of pUC19. The p55 coding SacI/Sall fragment was then further subcloned into the SacI/Sall site of a commercial pIC19R vector. After this procedure, the p55 coding sequence could be recloned into a unique BamHI site of the baculo-transfervector pVL941 (Summers et al., 1988). After a recombination event, the core protein was expressed by p55-recombinant baculo viruses in spodoptera frugiperda insect cells. The expression was analyzed by Western blot analysis (fig.3).

Example 4: Identification of premature p55 particles

[0038] p55-particle formation was characterized by sucrose sedimentation analysis and electron microscopy. Sucrose gradients were centrifuged at 28000 rpm for 150 minutes in an TST 41.14 rotor. 19 fractions were analysed by negative staining and Western blot analysis for p55 particles. Particles accumulated in fraction 17 at a sucrose density of 1.41 (fig.4). Analysis of spodoptera frugiperda cells by ultrathin section electron microscopy identified premature p55 particles budding from the cytoplasmic membran into the culture medium (fig.5).

Example 5: Computer assisted analysis of p55 secondary structure

[0039] To be able to insert selected epitopes derived from other reading frames than gag into the core protein in a rational manner, several p55 deletion constructs missing a stretch of 120 to 180 bp had to be established on DNA level. We decided, based on computer assisted analysis of the p55 secondary structure, to delete four 30 to 45 amino acids encoding sequences according to criteria of exposition of the respective regions on the surface of the p55 protein (fig. 6). All deletion mutants were derived from the pUC8p55 expression clone.

Example 6: Construction of the p55 deletion constructs

[0040] Basis of all four p55 deletion constructs was an especially designed polylinker supplementing the pUC8 derived polylinker. The new linker fragment contained all restriction sites for construction of four different p55 deletion clones, for direct expression in E. coli and for direct subcloning of the final constructs into different transfer vectors, designated for eukaryotic gene expression. The resulting pUC8 derivat was called plin8 (fig.7a).

Reference 1: p55 deletion construct-1 (plin8p55 1)

[0041] The development of the p55 deletion construct-1 based on the pUC19p55 clone described above. To delete two Accl sites, the p55 coding sequence was shortened for the 1100 bp 3'PstI fragment (pUC19p55BP). A 26bp linker fragment, containing two additional restriction sites (SacI/Xhol; fig. 7b) was inserted into the Clal/Accl sites of the N-terminal p55 coding sequence to achieve compatibility of the described construct to all following p55 deletion clones (pUC19p55BP 1). By this, a 129bp DNA fragment, encoding 43aa was deleted. Afterwards, the p55 coding sequence was completed by the 3'PstI/Sall fragment (pUC19p55 1). The resulting p55 deletion fragment-1 was subcloned into the plin8 vector (plin8p55 1) (fig.8).

Example 7: p55 deletion construct-2 (plin8p55 2)

[0042] The pUC8 derived 300bp BamHI/HindIII fragment, encoding an N-terminal part of p55 was cloned into the BamHI/HindIII site of plin8 (plin8p55BH). Afterwards, the 1283bp 3'Nsi/Sall fragment was inserted into the PstI/Sall site of plin8p55BH (plin8p55 2) (fig.9).

Example 8: Development of the deletion construct-3 (plin8p55 3)

[0043] A pUC8p55 derived BamHI/Sall fragment, encoding the complete HIV-1 core protein was subcloned into the BamHI/Sall sites of the described plin8 vector (plin8p55). To make the construct compatible to the oth-

er p55 deletion clones, a 27bp Xhol/SacI linker fragment was inserted into the PstI/Spel site of plin8p55. The resulting p55 deletion construct was renamed plin8p55 3 (fig.10).

Example 9: Development of the deletion construct-4 (plin8p55 4)

[0044] The complete pUC8p55 derived gag coding sequence was cloned into the BamHI/Sall site of plin8 (plin8p55). The 3'part (encoding aa 471-512) of the p55 coding sequence was amplified by polymerase chain reaction using a 61bp 5'primer containing five different restriction sites (BamHI, BgIII, Xhol, EcoRI, SacI) and a 21bp 3'primer containing a Hincl restriction site (fig. 7d). The amplified BgIII/Sall fragment was inserted into the BgIII/Sall site of the plin8p55 construct (fig.11). In the final plin8p55 4 clone, a 35aa encoding 105bp sequence was deleted.

Example 10: Compatibility of the four p55 deletion constructs

[0045] The developed p55 deletion constructs are compatible with regard to:

1. the insertion of foreign sequences. A defined sequence can be inserted into each of the four cassettes without changes in the reading frame.
2. the possibility to directly subclone the completed constructs into vectors appropriate for eukaryotic gene expression.

Example 11: Expression of the p55 deletion constructs in E. coli

[0046] All deletion constructs were expressed in E. coli JM109 after induction with 2mM IPTG. The expression products were verified by conventional Western blot analysis using anti p24 monoclonal antibodies.

Example 12: Insertion of selected epitopes of other reading frames

[0047]

1. The CD4 binding region of the HIV-1 gp120 and the fusogenic region of the gp41 were amplified by PCR reaction. 5'primers contained a Xhol-, 3'primers a SacI site to be able to insert amplified Xhol/ SacI fragments into the p55 deletion constructs (fig. 7e).
2. A designed consensus sequence of the gp120 major neutralizing epitope V3 was chemically synthesized and integrated into the Xhol/SacI site of the p55 deletion constructs (fig.7f).

Example 13: Expression of the completed p55/gp120 fusion proteins in E. coli

[0048] The p55/gp120 fusion products described in example 15 were expressed in E. coli after induction with 2 mM IPTG and analyzed by conventional Western blot analysis using anti-p24 monoclonal antibodies and a human serum pool (fig.12A).

Example 14: Verification of correct expression of an inserted epitope

[0049] The expression of the inserted gp120 major neutralizing epitope V3 was verified using a commercial monoclonal antibody directed to the V3 region of the HT-LVIIIB strain by conventional Western blot analysis. A polyclonal monospecific serum raised to the synthetic V3 consensus peptide also recognized the integrated epitope in Western blot analysis.

Example 15: Subcloning of the completed p55/gp120 constructs in eukaryotic expression vectors

[0050] The p55/gp120 constructs were subcloned (i) into the BgIII/Sall site of the vaccinia transfer vector pAvB (ii), into the unique BamHI site of the baculo transfer vector pVL941 and (iii) the XbaI/Sall site of the pMD vector for expression of the chimeric proteins in CHO cells.

Example 16: Expression of the completed p55/gp120 constructs in eukaryotic expression systems

[0051] After transfection and recombination of the hybrid genes into the viral DNA (Vaccinia, Baculo), the fusion proteins were expressed by recombinant vaccinia viruses in CV1- and by recombinant baculo viruses in Spodoptera frugiperda cells. The expression products were verified by conventional Western blot and immunofluorescence analysis with specific anti-p24 monoclonal antibodies (fig.14).

Example 17: Analysis of particle formation

[0052] Particle formation was studied by electron microscope using ultrathin sections of glutaraldehyd fixed cells infected with p55/gp120 recombinant vaccinia- or baculo viruses.

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Claims

Claims for the following Contracting States : ES, GR

1. A modified retroviral gag polypeptide which forms a particle wherein said gag polypeptide contains at least one amino acid sequence representing a non-gag polypeptide antigenic determinant, wherein the retrovirus is HIV-1, HIV-2 or SIV, wherein the gag polypeptide is p55, wherein said amino acid sequence substitutes (a) region(s) of said gag polypeptide, said region(s) being capable of presenting the inserted amino acid sequence to the immune system so as to elicit an immune response, or is (are) fused to the COOH-terminus of said gag polypeptide, and wherein said region is located at amino acid positions 99 to 154, 211 to 241, or 436 to 471 or at any combination thereof.
2. The modified retroviral gag polypeptide according to claim 1, wherein the non-gag polypeptide antigenic determinant is

- (a) at least a part of the CD4-binding domain of gp120;
- (b) at least a part of the variable region 3 of gp120; or
- (c) at least a part of the fusogenic region of gp41.

3. A DNA sequence encoding the modified retroviral gag polypeptide according to claim 1 or 2.
4. The DNA sequence according to claim 3, wherein the DNA sequence encoding the amino acid sequence is inserted via a linker.
5. A recombinant vector containing a DNA sequence according to claim 3 or 4.
6. A recombinant virus containing a DNA sequence according to claim 3 or 4.
7. The recombinant virus according to claim 6, which is a vaccinia virus.
8. A host organism which is transformed by a recom-

binant vector according to claim 5, or a recombinant virus according to claim 6 or 7.

- 5 9. A method for the production of a modified retroviral gag polypeptide according to claim 1 or 2, which comprises cultivating a host organism according to claim 8 under suitable conditions and recovering the expression product from the medium.
- 10 10. The method according to claim 9, wherein said host organism is an insect cell, preferably a Spodoptera frugiperda cell and wherein said virus with which said insect cell is transformed is a recombinant insect virus, preferably a baculo virus.
- 15 11. The method according to claim 9, wherein said host cell is an insect cell, preferably a Drosophila Schneider cell, and wherein said recombinant vector with which said insect cell is transformed is stably replicable in insect cells.
- 20 12. A vaccine containing a recombinant virus according to claim 6 or 7 or a modified retroviral gag polypeptide according to claim 1 or 2, optionally in combination with a pharmaceutically acceptable carrier and/or diluent.

Claims for the following Contracting State : GR

- 30 1. A modified retroviral gag polypeptide which forms a particle, wherein said gag polypeptide contains at least one amino acid sequence representing a non-gag polypeptide antigenic determinant, wherein the retrovirus is HIV-1, HIV-2 or SIV, wherein the gag polypeptide is p55, wherein said amino acid sequence substitutes (a) region(s) of said gag polypeptide, said region(s) being capable of presenting the inserted amino acid sequence to the immune system so as to elicit an immune response, or is (are) fused to the COOH-terminus of said gag polypeptide, and wherein said region is located at amino acid positions 99 to 154, 211 to 241, or 436 to 471 or at any combination thereof.
- 35 2. The modified retroviral gag polypeptide according to claim 1, wherein the non-gag polypeptide antigenic determinant is
 - (a) at least a part of the CD4-binding domain of gp120;
 - (b) at least a part of the variable region 3 of gp120; or
 - (c) at least a part of the fusogenic region of gp41.
- 40 3. A DNA sequence encoding the modified retroviral gag polypeptide according to claim 1 or 2.
- 45 4. The DNA sequence according to claim 3, wherein the DNA sequence encoding the amino acid sequence is inserted via a linker.
- 50 5. A recombinant vector containing a DNA sequence according to claim 3 or 4.
6. A recombinant virus containing a DNA sequence according to claim 3 or 4.
7. The recombinant virus according to claim 6, which is a vaccinia virus.
8. A host organism which is transformed by a recom-

4. The DNA sequence according to claim 3, wherein the DNA sequence encoding the amino acid sequence is inserted via a linker.

5. A recombinant vector containing a DNA sequence according to claim 3 or 4.

6. A recombinant virus containing a DNA sequence according to claim 3 or 4.

7. The recombinant virus according to claim 6, which is a vaccinia virus.

8. A host organism which is transformed by a recombinant vector according to claim 5, or a recombinant virus according to claim 6 or 7.

9. A method for the production of a modified retroviral gag polypeptide according to claim 1 or 2, which comprises cultivating a host organism according to claim 8 under suitable conditions and recovering the expression product from the medium.

10. The method according to claim 9, wherein said host organism is an insect cell, preferably a *Spodoptera frugiperda* cell and wherein said virus with which said insect cell is transformed is a recombinant insect virus, preferably a baculo virus.

11. The method according to claim 9, wherein said host cell is an insect cell, preferably a *Drosophila Schneider* cell, and wherein said recombinant vector with which said insect cell is transformed is stably replicable in insect cells.

12. Use of a recombinant virus according to claim 6 or 7 or a modified retroviral gag polypeptide according to claim 1 or 2, optionally in combination with a pharmaceutically acceptable carrier and/or diluent for the preparation of a vaccine.

Claims for the following Contracting State : ES

1. A method for the production of a DNA sequence encoding a modified retroviral gag polypeptide which forms a particle, wherein said gag polypeptide contains at least one amino acid sequence representing a non-gag polypeptide antigenic determinant wherein said gag polypeptide encoding DNA sequence is derived from any of the retroviruses HIV-1, HIV-2 or SIV, wherein said DNA sequence encoding said amino acid sequence substitutes (a) region(s) of said DNA sequence encoding said gag polypeptide, said region(s) being capable of presenting the amino acid sequence encoded by the inserted DNA sequence to the immune system so as to elicit an immune response, or wherein said

5. DNA sequence encoding said amino acid sequence is fused to the DNA sequence encoding the COOH-terminus of said gag polypeptide, and wherein said region is located at amino acid positions 99 to 154, 211 to 241, or 436 to 471 or at any combination thereof, said method comprising the insertion of at least one DNA sequence encoding a non-gag amino acid sequence into a DNA sequence encoding said gag polypeptide or the substitution of at least one codon of a DNA sequence encoding said gag polypeptide by a DNA sequence encoding a non-gag amino acid sequence.

15. 2. The method according to claim 1, wherein the DNA sequence encoding the additional amino acid sequence is inserted via a linker.

20. 3. The method according to claim 1 or 2, wherein the non-gag polypeptide antigenic determinant is

- (a) at least a part of the CD4-binding domain of gp120;
- (b) at least a part of the variable region 3 of gp120; or
- (c) at least a part of the fusogenic region of gp41.

25. 4. A method for the production of a recombinant vector comprising the insertion of a DNA sequence according to any one of claims 1 to 3 into a vector molecule.

30. 5. A method for the production of a recombinant virus comprising the steps of inserting a DNA sequence according to any one of claims 1 to 3 into a viral DNA molecule.

35. 6. The method according to claim 5, wherein the recombinant virus is a vaccinia virus.

40. 7. A method for the production of a host carrying a recombinant vector obtainable by a method according to claim 4 or a recombinant virus obtainable by a method according to claim 5 or 6, said method comprising the incorporation of said recombinant vector or said recombinant virus into a suitable host.

45. 8. A method for the production of a modified retroviral gag polypeptide which is encoded by a DNA sequence according to any one of claims 1 to 3, comprising the steps of cultivating a host obtainable by a method according to claim 7 under suitable conditions and recovering said polypeptide from the medium.

50. 9. A method according to claim 8, wherein the polypeptides form particles.

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10. The method according to claim 8 or 9, wherein said host organism is an insect cell, preferably a *Sophoptera frugiperda* cell and wherein said virus with which said insect cell is transformed is a recombinant insect virus, preferably a baculo virus.

11. The method according to claim 8 or 9, wherein said host cell is an insect cell, preferably a *Drosophila Schneider* cell, and wherein said recombinant vector with which said insect cell is transformed is stably replicable in insect cells.

Patentansprüche**Patentansprüche für folgende Vertragsstaaten : ES, GR**

1. Modifiziertes retrovirales gag-Polypeptid, das ein Partikel bildet, wobei das gag-Polypeptid mindestens eine Aminosäuresequenz enthält, die eine nicht-gag-Polypeptid-antigene Determinante darstellt, wobei das Retrovirus HIV-1, HIV-2 oder SIV ist, wobei das gag-Polypeptid p55 ist, wobei die Aminosäuresequenz (einen) Bereich(e) des gag-Polypeptids ersetzt, der (die) die eingefügte Aminosäuresequenz dem Immunsystem präsentieren kann/können, so daß eine Immunantwort hervorgerufen wird, oder der (die) mit dem COOH-Terminus des gag-Polypeptids verbunden ist/sind, und wobei der Bereich in den Aminosäurepositionen 99 bis 154, 211 bis 241 oder 436 bis 471 oder jeder Kombination davon liegt.

2. Modifiziertes retrovirales gag-Polypeptid nach Anspruch 1, wobei die nicht-gag-Polypeptid-antigene Determinante

(a) mindestens einen Teil der CD4-bindenden Domäne von gp120;
 (b) mindestens einen Teil des variablen Bereichs 3 von gp120; oder
 (c) mindestens einen Teil des fusogenen Bereichs von gp41

umfaßt.

3. DNA-Sequenz, die ein modifiziertes retrovirales gag-Polypeptid nach Anspruch 1 oder 2 codiert.

4. DNA-Sequenz nach Anspruch 3, wobei die DNA-Sequenz, die die Aminosäuresequenz codiert, über einen Linker eingefügt ist.

5. Rekombinanter Vektor, der die DNA-Sequenz nach Anspruch 3 oder 4 beinhaltet.

6. Rekombinantes Virus, das die DNA-Sequenz nach Anspruch 3 oder 4 beinhaltet.

7. Rekombinantes Virus nach Anspruch 6, das ein *Vaccinia*-Virus ist.

8. Wirtsorganismus, der mit einem rekombinanten Vektor nach Anspruch 5 oder einem rekombinanten Virus nach Anspruch 6 oder 7 transformiert ist.

9. Verfahren zur Herstellung eines modifizierten retroviralen gag-Polypeptids nach Anspruch 1 oder 2, das das Züchten eines Wirtsorganismus nach Anspruch 8 unter geeigneten Bedingungen und die Gewinnung des Expressionsprodukts aus dem Medium umfaßt.

10. Verfahren nach Anspruch 9, wobei der Wirtsorganismus eine Insektenzelle ist, vorzugsweise eine *Sophoptera frugiperda*-Zelle, und wobei das Virus, mit dem die Insektenzelle transformiert ist, ein rekombinantes Insektenvirus ist, vorzugsweise ein Baculovirus.

11. Verfahren nach Anspruch 9, wobei die Wirtszelle eine Insektenzelle ist, vorzugsweise eine *Drosophila Schneider*-Zelle, und wobei der rekombinante Vektor, mit dem die Insektenzelle transformiert ist, in den Insektenzellen stabil replizierbar ist.

12. Impfstoff, der ein rekombinantes Virus nach Anspruch 6 oder 7 oder ein modifiziertes retrovirales gag-Polypeptid nach Anspruch 1 oder 2 beinhaltet, gegebenenfalls in Kombination mit einem pharmazeutisch verträglichen Träger und/oder Verdünnungsmittel.

Patentansprüche für folgenden Vertragsstaat : GR

1. Modifiziertes retrovirales gag-Polypeptid, das ein Partikel bildet, wobei das gag-Polypeptid mindestens eine Aminosäuresequenz enthält, die eine nicht-gag-Polypeptid-antigene Determinante darstellt, wobei das Retrovirus HIV-1, HIV-2 oder SIV ist, wobei das gag-Polypeptid p55 ist, wobei die Aminosäuresequenz (einen) Bereich(e) des gag-Polypeptids ersetzt, der (die) die eingefügte Aminosäuresequenz dem Immunsystem präsentieren kann/können, so daß eine Immunantwort hervorgerufen wird, oder die mit dem COOH-Terminus des gag-Polypeptids verbunden ist/sind, und wobei der Bereich in den Aminosäurepositionen 99 bis 154, 211 bis 241, oder 436 bis 471 oder jeder Kombination davon liegt.

2. Modifiziertes retrovirales gag-Polypeptid nach Anspruch 1, wobei die nicht-gag-Polypeptid-antigene

Determinante

- (a) mindestens einen Teil der CD4-bindenden Domäne von gp120;
- (b) mindestens einen Teil des variablen Bereichs 3 von gp120; oder
- (c) mindestens einen Teil des fusogenen Bereichs von gp41

umfaßt.

3. DNA-Sequenz, die ein modifiziertes retrovirales gag-Polypeptid nach Anspruch 1 oder 2 codiert.

4. DNA-Sequenz nach Anspruch 3, wobei die DNA-Sequenz, die die Aminosäuresequenz codiert, über einen Linker eingefügt ist.

5. Rekombinanter Vektor, der die DNA-Sequenz nach Anspruch 3 oder 4 beinhaltet.

6. Rekombinantes Virus, das die DNA-Sequenz nach Anspruch 3 oder 4 beinhaltet.

7. Rekombinantes Virus nach Anspruch 6, das ein Vaccinia-Virus ist.

8. Wirtsorganismus, der mit einem rekombinanten Vektor nach Anspruch 5 oder einem rekombinanten Virus nach Anspruch 6 oder 7 transformiert ist.

9. Verfahren zur Herstellung eines modifizierten retroviralen gag-Polypeptids nach Anspruch 1 oder 2, das das Züchten eines Wirtsorganismus nach Anspruch 8 unter geeigneten Bedingungen und die Gewinnung des Expressionsprodukts aus dem Medium umfaßt.

10. Verfahren nach Anspruch 9, wobei der Wirtsorganismus eine Insektenzelle ist, vorzugsweise eine *Spodoptera frugiperda*-Zelle, und wobei das Virus, mit dem die Insektenzelle transformiert ist, ein rekombinantes Insektenvirus ist, vorzugsweise ein Baculovirus.

11. Verfahren nach Anspruch 9, wobei die Wirtszelle eine Insektenzelle ist, vorzugsweise eine *Drosophila Schneider*-Zelle, und wobei der rekombinante Vektor, mit dem die Insektenzelle transformiert ist, in den Insektenzellen stabil replizierbar ist.

12. Verwendung eines rekombinanten Virus nach Anspruch 6 oder 7 oder eines modifizierten retroviralen gag-Polypeptids nach Anspruch 1 oder 2, gegebenenfalls in Kombination mit einem pharmazeutisch verträglichen Träger und/oder Verdünnungsmittel zur Herstellung eines Vakzins.

Patentansprüche für folgenden Vertragsstaat : ES

1. Verfahren zur Herstellung einer DNA-Sequenz, die ein modifiziertes retrovirales gag-Polypeptid codiert, das ein Partikel formt, wobei das gag-Polypeptid mindestens eine Aminosäuresequenz enthält, die eine nicht-gag-Polypeptid-antigene Determinante darstellt, wobei die gag-Polypeptid-codierende DNA-Sequenz von einem der Retroviren HIV-1, HIV-2 oder SIV stammt, wobei die die Aminosäuresequenz codierende DNA-Sequenz (einen) Bereich(e) der DNA-Sequenz ersetzt, der (die) das gag-Polypeptid codiert, wobei der (die) Bereich(e) dem Immunsystem die von der eingefügten DNA-Sequenz codierte Aminosäuresequenz präsentieren kann/können, so daß eine Immunantwort hervorgerufen wird, oder wobei die die Aminosäuresequenz codierende DNA-Sequenz mit der den COOH-Terminus des gag-Polypeptids codierenden DNA-Sequenz verbunden ist, und wobei der Bereich in den Aminosäurepositionen 99 bis 154, 211 bis 241 oder 436 bis 471 oder jeder Kombination davon liegt, wobei das Verfahren die Einfügung mindestens einer DNA-Sequenz, die eine nicht-gag-Aminosäuresequenz codiert, in eine das gag-Polypeptid codierende DNA-Sequenz oder den Austausch von mindestens einem Codon einer das gag-Polypeptid codierenden DNA-Sequenz durch eine eine nicht-gag-Aminosäuresequenz codierende DNA-Sequenz umfaßt.
2. Verfahren nach Anspruch 1, wobei die die zusätzliche Aminosäuresequenz codierende DNA-Sequenz über einen Linker eingefügt ist.
3. Verfahren nach Anspruch 1 oder 2, wobei die nicht-gag-Polypeptid-antigene Determinante
 - (a) mindestens ein Teil der CD4-bindenden Domäne von gp120;
 - (b) mindestens ein Teil des variablen Bereichs 3 von gp120; oder
 - (c) mindestens ein Teil des fusogenen Bereichs von gp41
4. Verfahren zur Herstellung eines rekombinanten Vektors, das das Einfügen einer DNA-Sequenz nach einem der Ansprüche 1 bis 3 in ein Vektormolekül umfaßt.
5. Verfahren zur Herstellung eines rekombinanten Virus, das die Schritte zum Einfügen einer DNA-Sequenz nach einem der Ansprüche 1 bis 3 in ein virales DNA-Molekül umfaßt.
6. Verfahren nach Anspruch 5, wobei das rekombi-

nante Virus ein Vaccinia-Virus ist.

7. Verfahren zur Herstellung eines Wirts, der einen durch ein Verfahren nach Anspruch 4 erhältlichen rekombinanten Vektor oder ein durch ein Verfahren nach Anspruch 5 oder 6 erhältliches rekombinantes Virus trägt, wobei das Verfahren das Einbringen des rekombinanten Vektors oder des rekombinanten Virus in einen geeigneten Wirt umfasst.
8. Verfahren zur Herstellung eines modifizierten retroviralen gag-Polypeptids, das durch eine DNA-Sequenz nach einem der Ansprüche 1 bis 3 codiert wird, umfassend die Schritte der Züchtung eines durch ein Verfahren nach Anspruch 7 erhältlichen Wirts unter geeigneten Bedingungen und die Gewinnung des Polypeptids aus dem Medium.
9. Verfahren nach Anspruch 8, wobei die Polypeptide Partikel bilden.
10. Verfahren nach Anspruch 8 oder 9, wobei der Wirtsorganismus eine Insektenzelle, vorzugsweise eine *Spodoptera frugiperda*-Zelle und wobei das Virus, mit dem die Insektenzelle transformiert ist, ein rekombinantes Insektenvirus ist, vorzugsweise ein Baculovirus.
11. Verfahren nach Anspruch 8 oder 9, wobei die Wirtszelle eine Insektenzelle ist, bevorzugt eine *Drosophila Schneider*-Zelle, und wobei der rekombinante Vektor, mit dem die Insektenzelle transformiert ist, in den Insektenzellen stabil replizierbar ist.

Revendications

Revendications pour les Etats contractants suivants : ES, GR

1. Polypeptide gag rétroviral modifié constituant une particule, dans lequel ledit polypeptide gag contient au moins une séquence d'aminoacides représentant un déterminant antigénique polypeptidique non-gag, dans lequel le rétrovirus est VIH-1, VIH-2 ou SIV, dans lequel le polypeptide gag est p55, dans lequel ladite séquence d'aminoacides remplace une (des) région(s) dudit polypeptide gag, ladite (lesdites) région(s) étant capable(s) de présenter au système immunitaire la séquence d'aminoacides insérée, de manière à susciter une réponse immunitaire, ou étant soudée(s) à l'extrémité COOH-terminale dudit polypeptide gag, et dans lequel ladite région est localisée aux positions d'aminoacides 99 à 154, 211 à 241, ou 436 à 471, ou à une combinaison quelconque de celles-ci.

2. Polypeptide gag rétroviral modifié, selon la revendication 1, dans lequel le déterminant antigénique polypeptidique non-gag est

- 5 (a) au moins une partie du domaine de liaison à CD4 de gp120;
- (b) au moins une partie de la région variable 3 de gp120; ou
- (c) au moins une partie de la région fusiogène de gp41.

- 10 3. Séquence d'ADN codant pour le polypeptide gag rétroviral modifié, selon la revendication 1 ou 2.

- 15 4. Séquence d'ADN selon la revendication 3, dans laquelle la séquence d'ADN codant pour la séquence d'aminoacides est insérée par l'intermédiaire d'un lieu.

- 20 5. Vecteur recombinant contenant une séquence d'ADN selon la revendication 3 ou 4.

- 25 6. Virus recombinant contenant une séquence d'ADN selon la revendication 3 ou 4.

- 30 7. Virus recombinant selon la revendication 6, qui est un virus de la vaccine.

- 35 8. Organisme hôte qui est transformé par un vecteur recombinant selon la revendication 5 ou un virus recombinant selon la revendication 6 ou 7.

- 40 9. Procédé pour la production d'un polypeptide gag rétroviral modifié, selon la revendication 1 ou 2, comprenant la culture d'un organisme hôte selon la revendication 8, dans des conditions appropriées, et la récupération du produit d'expression à partir du milieu.

- 45 10. Procédé selon la revendication 9, dans lequel ledit organisme hôte est une cellule d'insecte, de préférence une cellule de *Spodoptera frugiperda*, et dans lequel ledit virus par lequel ladite cellule d'insecte est transformée est un virus recombinant d'insecte, de préférence un baculovirus.

- 50 11. Procédé selon la revendication 9, dans lequel la cellule hôte est une cellule d'insecte, de préférence une cellule de *Schneider* de *Drosophila*, et dans lequel ledit vecteur recombinant par lequel ladite cellule d'insecte est transformée est répliable de façon stable dans des cellules d'insectes.

- 55 12. Vaccin contenant un virus recombinant selon la revendication 6 ou 7, ou un polypeptide gag rétroviral modifié, selon la revendication 1 ou 2, éventuellement en association avec un véhicule et/ou diluant pharmaceutiquement acceptable.

Revendications pour l'Etat contractant suivant : GR

1. Polypeptide gag rétroviral modifié constituant une particule, dans lequel ledit polypeptide gag contient au moins une séquence d'aminoacides représentant un déterminant antigénique polypeptidique non-gag, dans lequel le rétrovirus est VIH-1, VIH-2 ou SIV, dans lequel le polypeptide gag est p55, dans lequel ladite séquence d'aminoacides remplace une (des) région(s) dudit polypeptide gag, ladite (lesdites) région(s) étant capable(s) de présenter au système immunitaire la séquence d'aminoacides insérée, de manière à susciter une réponse immunitaire, ou étant soudée(s) à l'extrémité COOH-terminale dudit polypeptide gag, et dans lequel ladite région est localisée aux positions d'aminoacides 99 à 154, 211 à 241, ou 436 à 471, ou à une association quelconque de celles-ci.

2. Polypeptide gag rétroviral modifié, selon la revendication 1, dans lequel le déterminant antigénique polypeptidique non-gag est

- (a) au moins une partie du domaine de liaison à CD4 de gp120;
- (b) au moins une partie de la région variable 3 de gp120; ou
- (c) au moins une partie de la région fusiogène de gp41.

3. Séquence d'ADN codant pour le polypeptide gag rétroviral modifié, selon la revendication 1 ou 2.

4. Séquence d'ADN selon la revendication 3, dans laquelle la séquence d'ADN codant pour la séquence d'aminoacides est insérée par l'intermédiaire d'un lieu.

5. Vecteur recombinant contenant une séquence d'ADN selon la revendication 3 ou 4.

6. Virus recombinant contenant une séquence d'ADN selon la revendication 3 ou 4.

7. Virus recombinant selon la revendication 6, qui est un virus de la vaccine.

8. Organisme hôte qui est transformé par un vecteur recombinant selon la revendication 5 ou un virus recombinant selon la revendication 6 ou 7.

9. Procédé pour la production d'un polypeptide gag rétroviral modifié, selon la revendication 1 ou 2, comprenant la culture d'un organisme hôte selon la revendication 8, dans des conditions appropriées, et la récupération du produit d'expression à partir du milieu.

5 10. Procédé selon la revendication 9, dans lequel ledit organisme hôte est une cellule d'insecte, de préférence une cellule de *Spodoptera frugiperda*, et dans lequel ledit virus par lequel ladite cellule d'insecte est transformée est un virus recombinant d'insecte, de préférence un baculovirus.

10 11. Procédé selon la revendication 9, dans lequel la cellule hôte est une cellule d'insecte, de préférence une cellule de Schneider de *Drosophila*, et dans lequel ledit vecteur recombinant par lequel ladite cellule d'insecte est transformée est répliable de façon stable dans des cellules d'insectes.

15 12. Utilisation d'un virus recombinant selon la revendication 6 ou 7, ou d'un polypeptide gag rétroviral modifié, selon la revendication 1 ou 2, éventuellement en association avec un véhicule et/ou diluant pharmaceutiquement acceptable, pour la fabrication d'un vaccin.

Revendications pour l'Etat contractant suivant : ES

25 1. Procédé pour la production d'une séquence d'ADN codant pour un polypeptide gag rétroviral modifié constituant une particule, dans lequel ledit polypeptide gag contient au moins une séquence d'aminoacides représentant un déterminant antigénique polypeptidique non-gag, dans lequel ladite séquence d'ADN codant pour un polypeptide gag est issue de l'un quelconque des rétrovirus VIH-1, VIH-2 et SIV, dans lequel ladite séquence d'ADN codant pour la-dite séquence d'aminoacides remplace une (des) région(s) de ladite séquence d'ADN codant pour le-dit polypeptide gag, ladite (lesdites) région(s) étant capable(s) de présenter au système immunitaire la séquence d'aminoacides codée par la séquence d'ADN insérée, de manière à susciter une réponse immunitaire, ou dans lequel ladite séquence d'ADN codant pour ladite séquence d'aminoacides est soudée à la séquence d'ADN codant pour l'extrémité COOH-terminale dudit polypeptide gag, et dans lequel ladite région est localisée aux positions d'aminoacides 99 à 154, 211 à 241 ou 436 à 471 ou à une association quelconque de celles-ci, ledit procédé comprenant l'insertion d'au moins une séquence d'ADN codant pour une séquence d'aminoacides non-gag dans une séquence d'ADN codant pour ledit polypeptide gag, ou le remplacement d'au moins un codon d'une séquence d'ADN codant pour ledit polypeptide gag par une séquence d'ADN codant pour une séquence d'aminoacides non-gag.

30 2. Procédé selon la revendication 1, dans lequel la séquence d'ADN codant pour la séquence d'aminoacides supplémentaire est insérée par l'intermédiaire d'un lieu.

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3. Procédé selon la revendication 1 ou 2, dans lequel le déterminant antigénique polypeptidique non-gag est

(a) au moins une partie du domaine de liaison à CD4 de gp120; 5
 (b) au moins une partie de la région variable 3 de gp120; ou
 (c) au moins une partie de la région fusiogène de gp41. 10

4. Procédé pour la production d'un vecteur recombinant, comprenant l'insertion d'une séquence d'ADN selon l'une quelconque des revendications 1 à 3, dans une molécule vectrice. 15

5. Procédé pour la production d'un virus recombinant, comprenant les étapes d'insertion d'une séquence d'ADN selon l'une quelconque des revendications 1 à 3, dans une molécule d'ADN viral. 20

6. Procédé selon la revendication 5, dans lequel le virus recombinant est un virus de la vaccine.

7. Procédé pour la production d'un hôte portant un vecteur recombinant pouvant être obtenu par un procédé selon la revendication 4 ou un virus recombinant pouvant être obtenu par un procédé selon la revendication 5 ou 6, ledit procédé comprenant l'incorporation dudit vecteur recombinant ou dudit virus recombinant dans un hôte approprié. 25 30

8. Procédé pour la production d'un polypeptide gag rétroviral modifié qui est codé par une séquence d'ADN selon l'une quelconque des revendications 1 à 3, comprenant les étapes de culture d'un hôte pouvant être obtenu par un procédé selon la revendication 7, dans des conditions appropriées, et la récupération dudit polypeptide à partir du milieu. 35 40

9. Procédé selon la revendication 8, dans lequel les polypeptides constituent des particules.

10. Procédé selon la revendication 8 ou 9, dans lequel ledit organisme hôte est une cellule d'insecte, de préférence une cellule de *Spodoptera frugiperda*, et dans lequel ledit virus par lequel ladite cellule d'insecte est transformée est un virus recombinant d'insecte, de préférence un baculovirus. 45 50

11. Procédé selon la revendication 8 ou 9, dans lequel ladite cellule hôte est une cellule d'insecte, de préférence une cellule de Schneider de *Drosophila*, et dans lequel ledit vecteur recombinant par lequel ladite cellule d'insecte est transformée est répliable de façon stable dans des cellules d'insectes. 55